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SOME PROPERTIES OF THE PRESYNAPTIC NERVE TERMINALS IN A MAMMALIAN SYMPATHETIC GANGLION

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SUMMARY

1. Superior cervical ganglia of adult rats were excised and maintained *in vitro* in stable conditions. Potentials were recorded with external electrodes. After transmission was blocked by mecamylamine, a small potential change was recorded from the rostral area of the ganglion in response to preganglionic stimulation.

2. This electrical response was identified as the presynaptic action potential recorded from the nerve terminals by a number of criteria based on histological and physiological considerations including the disappearance of the spike in a glucose free solution. As shown by Nicolescu, Dolivo, Rouiller & Foroglou-Kerameus (1966) on the same preparation this condition causes an irreversible and selective lesion of the presynaptic nerve endings.

3. A suitable concentration of mecamylamine permitted the presynaptic response and the excitatory post-synaptic potential (EPSP) to be recorded simultaneously. As the stimulus was increased, the EPSP increased linearly with the amplitude of the presynaptic response.

4. After replacement of potassium ions in the bathing solution by caesium and during the early phase of post-tetanic facilitation there was an increase in the presynaptic response accompanied by a disproportionate increase in the EPSP.

5. No changes in the presynaptic response were found in the presence of the following drugs, all of which depressed the EPSP: acetylcholine, hemicholinium, curare, further doses of ganglion-blocking agents, and high Mg^{2+} and low Ca^{2+} concentrations.

6. Ouabain ($4{\cdot}5 \times 10^{-4}$ M) reversibly decreased the amplitude of the presynaptic response and increased the spontaneous release of transmitter. The EPSP was at first enhanced and then depressed.

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INTRODUCTION

In the superior cervical ganglion of the rat, presynaptic axons end in a rich network of branched and tortuous terminals of 0·1–0·3 μ in diameter with local enlargements to 0·5 μ. Most of the synapses occur not at the end of the fibres but as axo-dendritic *boutons de passage* (Taxi, 1961; Forssmann, 1964; see also Elfvin, 1963). In this paper a method is described for external recording of the 'mass' electrical activity of this complex in response to preganglionic nerve stimulation. The method has been used to investigate the properties of the presynaptic response and the relation between it and the post-synaptic response (EPSP) under various conditions.

Partial and brief communications of these findings have been published elsewhere (Dunant, 1968, 1969; Dunant & Dolivo, 1968*a*, *b*). The work on ouabain was initiated in collaboration with F. de Ribaupierre (de Ribaupierre, Dunant, Dolivo & Foroglou-Kerameus, 1968).

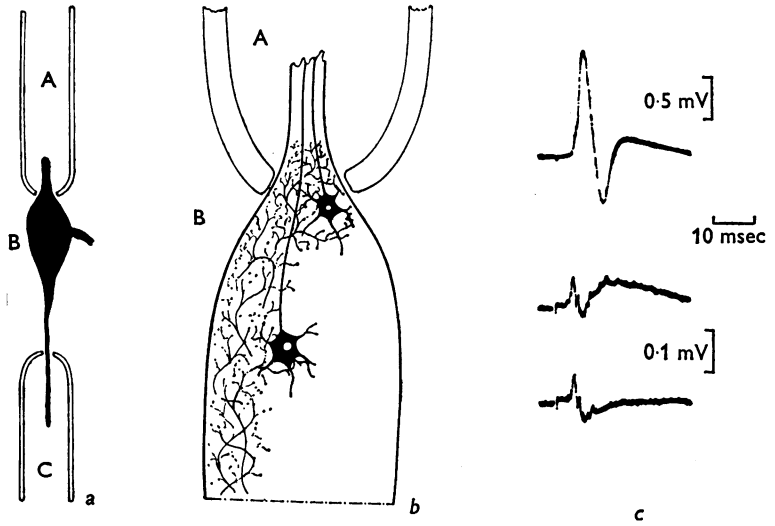

Fig. 1. Recording of the presynaptic response from the superior cervical ganglion of the rat. *a*, Experimental device. The cervical trunk was stimulated (or its potentials recorded) between B and C. The responses of the ganglion were recorded from the rostral area between A and B. *b*, Highly diagrammatic scheme of the rostral area. *c*, responses of the rostral area to supramaximal stimulation of the preganglionic trunk in a standard Krebs solution (top record), or 45 min after application of mecamylamine $2{\cdot}5 \times 10^{-5}$ M (middle record) and 10^{-4} M (bottom record). Further descriptions in the text. Note the slight deflexion caused by 'the initial response' at the rising phase of the unblocked response. In this picture and the following ones the records have been redrawn from the original photographs.

METHODS

Superior cervical ganglia of Wistar rats were excised under urethane anaesthesia and put in a chamber containing oxygenated Krebs solution. The internal carotid (post-ganglionic) nerve was gently pulled by a fine thread into a glass tube in such way that the enlarged part of the rostral region of the ganglion closed the tube opening as shown in Fig. 1*a*. Two Ag-AgCl wires, one of them dipped in the solution inside the tube (A), the other one outside (B), were used for recording. The resistance of this device was low (20–50 kΩ). The preganglionic nerve was pulled into a similar tube to allow stimulation (C).

Fig. 1*b* illustrates the arrangement of the recording system with respect to the ganglion. The position of the tubes opening was quite critical for obtaining satisfactory records. When properly placed, it was possible to stimulate the ganglion, to record its responses or to change the solution without altering the position of electrodes for many hours.

Action potentials of the preganglionic trunk itself were occasionally recorded with an intermediate electrode placed between the tube C and the ganglion (for details see Dunant, 1967).

Composition of the Krebs solution (in mM): NaCl, 136; KCl, 5·6; $NaHCO_3$, 16·2; NaH_2PO_4, 1·2; $CaCl_2$, 2·2; $MgCl_2$, 1·2 and glucose 5·5. The solution was equilibrated with 95 % O_2 and 5 % CO_2. All experiments were carried out at 37° C.

Hexamethonium-Cl and pentolinum-tartrate were a generous gift from Professor Paton, Oxford, and D-tubocurarine (Burroughs Wellcome, London) from Pharmacolor AG, Basle. Mecamylamine was obtained from Merck, Sharp and Dohme, acetylcholine perchlorate from British Drug Houses, hemicholinium from Aldrich and Co. and ouabain, G-strophanthins, from Merck AG.

RESULTS

Recording and identification of the presynaptic action potential. Fig. 1*c* (top record) shows an action potential recorded from the rostral area of the ganglion after an isolated supramaximal stimulus to the preganglionic nerve.

When $2{\cdot}5 \times 10^{-5}$ M mecamylamine was added to the Krebs solution the amplitude of the ganglion response was greatly reduced (Fig. 1*c*, middle trace). The record now showed two components. The early component was a diphasic response, positive-negative with respect to the inside of the tube. The late component was mainly positive and consisted of a slow rising phase, followed by a much slower decay. It was recognized as the EPSP since, in contrast to the initial response, its amplitude was a function of mecamylamine concentration (Fig. 1*c*, bottom record).

The amplitude on the initial response was increased by raising the intensity of the shocks to the preganglionic trunk. The increases were directly proportional to those of the B_2 potential that could be recorded with an intermediate electrode placed on the nerve (see Methods). The B_2 potential is related to the largest group of preganglionic fibres activating the rostral region of the ganglion (Dunant, 1967). Action potentials could

be conducted in both directions between this region and the preganglionic trunk but the velocity of conduction was slower than in the nerve itself.

Stimuli were applied and records were made from various positions on the ganglion and its nerves. The initial response could not be recorded from the post-ganglionic nerves at distance of 2 mm or more from the ganglion. Neither did stimuli applied there elicit any antidromic response in the preganglionic trunk. Both were only observed when the electrode reached the extreme top of the ganglion. In fact it was possible from histological preparations (made by Mme Foroglou-Kerameus) to establish a good correlation between the positions where the initial response was recorded and the presence of nerve cells and presynaptic terminals.

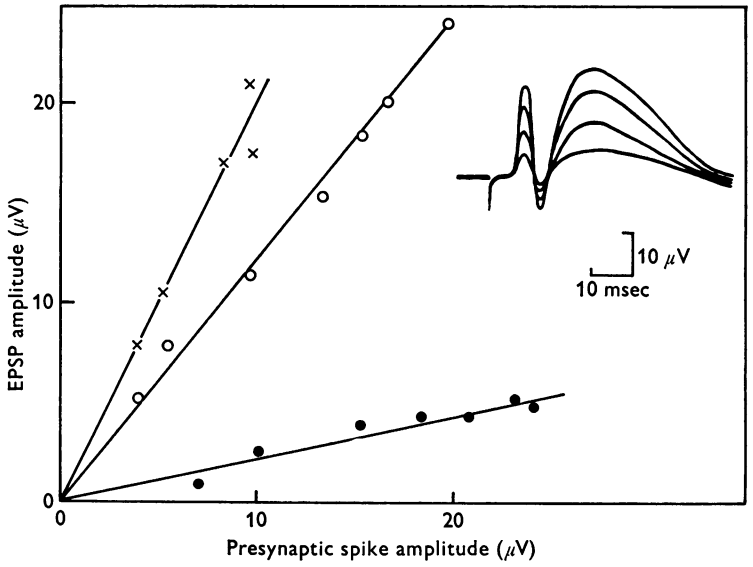

Fig. 2. Relationships between the pre- and post-synaptic responses when the preganglionic trunk was given shocks on increasing intensity. The relation is shown for three preparations. The responses of one of them have been redrawn by superimposition in inset. Mecamylamine $2{\cdot}5 \times 10^{-5}$ M.

When the preganglionic trunk was excited with increasing intensity, more B_2 fibres were activated, the EPSP also increased, its amplitude being directly proportional to that of the presynaptic spike (Fig. 2). Similar results were obtained from twelve preparations. The slope of the line varied from ganglion to ganglion presumably because of differences in anatomical factors and recording conditions.

The initial response was found to be very sensitive to glucose. One hour after exposure of the preparation to glucose-free solution, the amplitude

of the initial response was reduced to half its original value; after 3 hr, the response completely disappeared. The EPSP vanished with the same time course. These effects were irreversible. The disappearance of the initial response in the present experiments corresponds well with the time course with which glucose lack has been shown to cause a selective lesion of the presynaptic terminals in the same preparation (Nicolescu *et al.* 1966). All of the terminals were emptied of their synaptic vesicles and their neuroplasm was vacuolated. In contrast the surrounding nerve and satellite cells remained undamaged, the ganglia were still able to respond to acetylcholine and the nerve trunks to conduct action potentials for hours. Thus, at this stage, the lesion was confined to presynaptic terminals.

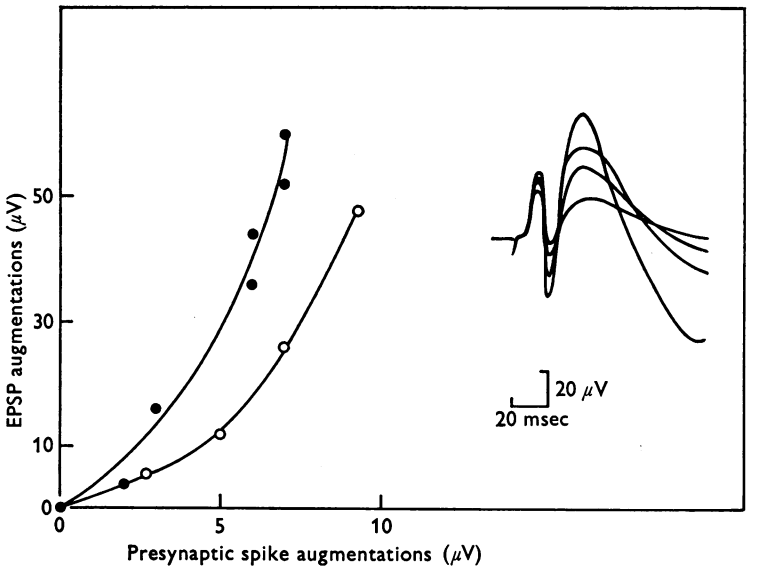

Fig. 3. Relationships between the pre- and post-synaptic potentials under conditions of post-tetanic facilitation. The augmentations of the potentials are shown for two preparations. Note the difference in the scales. In inset, superimposed drawings of the presynaptic spikes and EPSPs recorded from the same preparation at rest (smallest deflexion) and 2 sec after 10 sec trains of stimuli at 10/sec, 20/sec and 40/sec. Mecamylamine $2{\cdot}5 \times 10^{-5}$ M.

These results indicate that the initial response is a compound *presynaptic action potential* recorded from the terminal network of the preganglionic B_2 fibres.

After-effects of stimulation on the presynaptic response and EPSP. In sympathetic ganglia, facilitation involves short- and long-lasting changes (Larrabee & Bronk, 1947). The later were not related to any modification of the presynaptic spike and were determined by the concentration of

external calcium prevailing at the time of the conditioning stimulation (Dunant & Dolivo, 1968*b*; Dunant, 1969).

The early modifications were investigated in the presence of $2{\cdot}5 \times 10^{-5}$ M mecamylamine. The preganglionic trunk was stimulated with trains of conditioning shocks of 10 sec duration at various frequencies and the response to a test volley was recorded 2 sec after the end of the trains. In this response the amplitude of the presynaptic spike increased as the frequency was raised in the conditioning stimulation. The corresponding changes of the EPSP, however, were much larger than those of the presynaptic action potential. The relationship between their amplitudes was approximated by a logarithmic function, the slope of which was very steep (Fig. 3). The intensity of stimulation was supramaximal all along and intervals of 5 min separated the successive conditioning trains. The action potentials of the preganglionic nerve were also recorded in a few cases by an intermediate electrode. The amplitude of the B_2 spike was not modified by a single conditioning stimulus, a slight decrease was sometimes observed after repetitive activation.

Effects of caesium ions. Caesium ions cause a large potentiation of both excitatory and inhibitory post-synaptic potentials (EPSP and IPSP) at the lobster neuromuscular junction (Gainer, Reuben & Grundfest, 1967). Ginsborg & Hamilton (1968) suggested that caesium would enter the nerve terminals modifying the shape of their action potential.

When a ganglion was incubated in a modified solution containing no KCl but an equivalent concentration (5·6 mM) of CsCl, its action potential was augmented more than twofold. The effect had a slow time course reaching a maximum in about an hour. Later it declined. The removal of K^+ by itself, without replacement by Cs^+, caused only a very small increase in the action potential amplitude which appeared very rapidly. The changes observed were thus due to Cs ions. The effect could be slowly reversed by returning in a standard Krebs solution within the first 2 hr.

The presynaptic mechanisms involved in the Cs^+ effect were further investigated in the presence of mecamylamine ($2{\cdot}5$–10×10^{-5} M). The presynaptic response and EPSP were measured before and after substitution of Cs^+ for K^+. During the onset of the Cs^+ effect, the presynaptic spike increased in both amplitude and duration. The relative changes of the EPSP were much larger. During the first half hour the relation between EPSP and presynaptic response was sigmoidal (Fig. 4). Later in the experiment the EPSP overlapped the presynaptic spike and large action potentials reappeared, the blockade by mecamylamine presumably being overcome. The potentiation of the presynaptic response was best observed in those preparations where transmission was completely blocked by a high concentration of mecamylamine (4×10^{-4} M). Even so the EPSP reappeared

after some time, and increased in a sigmoidal relation to the augmentation of the presynaptic spike. The relation was observed from ten blocked ganglia. The time course of the Cs^+ effect was the same in the presence or the absence of the ganglion-blocking drug.

Hancock & Volle (1968) have shown that Cs^+ causes cat ganglia to discharge asynchronously. The effect was confirmed in these present experiments. The firing could be suppressed by mecamylamine or by changing to a new solution which contained low Ca^{2+} and high Mg^{2+} concentrations. It did not occur in deafferented ganglia.

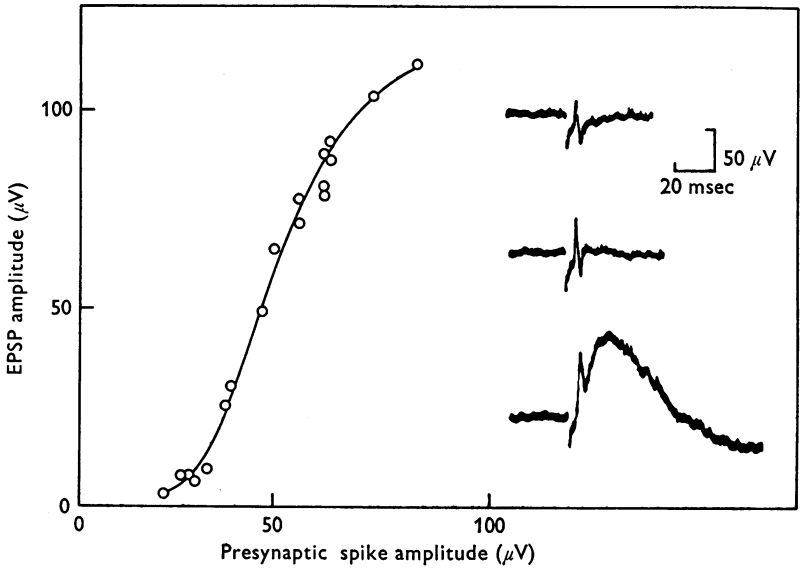

Fig. 4. Relationships between the pre- and post-synaptic responses during the onset of the Cs effect. In the inset, records taken before, and 10 and 30 min after Cs application. Mecamylamine 10^{-4} M throughout the experiment.

Pharmacological properties of the presynaptic response. In a small number of experiments, the presynaptic spike was also observed with ganglion-blocking agents other than mecamylamine, namely hexamethonium (5×10^{-4} M), pentolinum (2×10^{-4} M), curare (D-tubocurarine, 6×10^{-4} M) or a modified solution containing 0·55 mM-$CaCl_2$ and 6 mM-$MgCl_2$. Addition of an excess of *curare* or *ganglion-blocking* agent did not modify the size and shape of the presynaptic spike. Application of *hemicholinium* (2×10^{-4} M) did not alter the spike even after long preganglionic stimulation resulting in a complete disappearance of the EPSP.

Acetylcholine (ACh) was also tested in concentrations up 4×10^{-3} M on

six ganglia. The potentials were measured 2 and 5 min after ACh application. The EPSP was depressed as a function of ACh concentration but no alteration of the presynaptic spike was observed. Using a different method of recording, Koketsu & Nishi (1968) have reported a transient reduction in the amplitude of the presynaptic response. An effect which lasted less than 2 min would not have been detected in the present experiments.

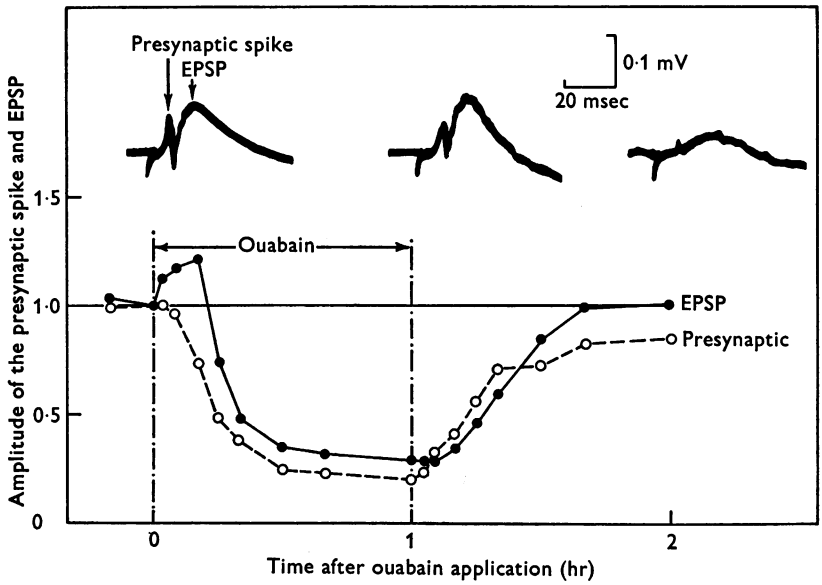

Fig. 5. Effects of ouabain ($4{\cdot}5 \times 10^{-4}$ M) on the presynaptic spike and EPSP. The amplitude of the potentials are expressed as relative values of the controls tested in the absence of ouabain. They were measured simultaneously from the same ganglion. In inset, records taken before, and then 6 and 25 min after ouabain application. Mecamylamine $2{\cdot}5 \times 10^{-5}$ M throughout the experiment.

At the neuromuscular junction, *ouabain* is known to cause a transient increase in the spontaneous release of transmitter followed by the blockade of the transmission (Elmqvist & Feldman, 1965; Birks & Cohen, 1968). Since the effect can be due to depolarization of presynaptic nerve terminals it was investigated on sympathetic ganglia.

Ouabain ($4{\cdot}5 \times 10^{-4}$ M) when added to Krebs solution blocked synaptic transmission in about 20 min. The effect was reversible. At this concentration ouabain caused only a small impairment of axonal conduction in the pre- and post-ganglionic nerves. Between 7 and 20 min after the application of ouabain, asynchronous activity was recorded from the post-ganglionic nerves even in the absence of any anticholinesterase drug. The

firing reached a maximum at 14–16 min. It was suppressed or prevented by D-tubocurarine (4×10^{-4} M) or mecamylamine (10^{-5}–10^{-4} M).

The effects of ouabain on the presynaptic spike and EPSP were investigated on seven ganglia in the presence of mecamylamine ($2{\cdot}5 \times 10^{-5}$ M). As shown in Fig. 5, the drug strongly depressed the response of presynaptic nerve terminals. Its amplitude was reduced to 0·23 of its initial value (S.E. = 0·05; n = 7).

The EPSP was consistently affected by ouabain in a quite different manner (Fig. 5). Its amplitude was at first increased reaching 1·30 times its original value (S.E. = 0·07; n = 7) $10 \pm 1{\cdot}5$ min after ouabain was given. The EPSP then rapidly fell towards a steady level which was 0·38 (S.E. = 0·05; n = 7) of the original value.

DISCUSSION

External electrodes were used in the present work to record the 'mass' electrical activity of the presynaptic nerve terminals. These records must be evaluated carefully since the response is due to the activity of numerous elements. Any modification of its amplitude can be due to changes in the number of active terminals or in the configuration of their individual action potential.

The relationship between the amplitude of the presynaptic response and the EPSP was linear when the number of active terminals was increased by raising the intensity of the preganglionic stimulation. The same relationship became sigmoidal either during exposure to caesium or during a short interval following a conditioning train of repetitive stimulation. This new relationship could be a consequence of a change in either the amplitude or the duration of the action potential of the individual nerve endings (Hagiwara & Tasaki, 1958; Katz & Miledi, 1967). Intracellular caesium has been shown in single axons to increase the duration of the action potential (Baker, Hodgkin & Shaw, 1962; Sjodin, 1966). Ginsborg & Hamilton (1968) suggested that the augmentation of transmitter release which follows extracellular application of caesium to the frog neuromuscular junction might be a consequence of increased action potential duration. Similarly an increase in the duration of the individual action potentials may explain post-tetanic changes reported here; such an increase was observed in other fibres of small diameter (Ritchie & Straub, 1956).

Depolarization of the nerve terminals by ouabain may account for the depression of the presynaptic response and the spontaneous release of transmitter. At the rat neuromuscular junction, the increased spontaneous release follows an identical time course (Elmqvist & Feldman, 1965). The initial enhancement of the post-synaptic response by ouabain cannot be

explained by presynaptic depolarization. This effect was also observed at the frog neuromuscular junction (Birks & Cohen, 1968) and might be due to increased mobilization of calcium ions.

Under a variety of circumstances, the electrical response of nerve terminals was found to differ from that of their parent axons. The cases in which the electrical activity of the preganglionic nerve terminals can be modified seems to reflect their large surface to volume ratio and their high metabolic requirements depending on external supply.

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